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Predictive value of liver tissue flow in assessment of the viability of liver grafts after extended preservation in pigs

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Abstract. The crucial damage in cold storage of liver allografts is to the hepatic sinusoidal lining (microcirculation). Using different solutions, we studied whether determinations of graft tissue flow were valuable in estimating the viability of liver grafts. Twenty-three pairs of female pigs underwent orthotopic liver transplantation and were assigned to five groups according to the cold preservation time or solutions used: in group I the liver grafts were stored in Euro-Collins solution (EC) for 4 h ($n = 3$), in group II the grafts were stored in EC for 12 h ($n = 5$), in group III the donor was pretreated with azathioprine (AZA), 1 mg/kg per day, orally (PO) for 3 days before harvesting and the graft was implanted after 12 h cold storage with EC ($n = 6$), in group IV the graft was stored in modified University of Wisconsin solution (mUW) for 4 h ($n = 3$), and in group V the graft was stored in mUW for 24 h ($n = 6$). Liver tissue blood flow (LTBF) was measured, using a laser doppler device, at 60 min after recirculation of the graft. In the case of EC preservation, LTBF (ml/100 g of liver tissue per min) correlated well with 4-day survival: 21.2 ± 3.0 ml/100 g of tissue per min mean ± SD, in group I (3/3, 100%); 10.0 ± 2.8 ml/100 g of tissue per min in group II (0/5, 0%); and 19.1 ± 3.4 ml/100 g of tissue per min in group III (5/6, 83.3%) ($P < 0.05$, group II vs I and III). All grafts with LTBF of more than 15 ml/100 g tissue per min functioned well. However, changes in microcirculation of the mUW-stored livers did not correlate with early function of the graft: 23.0 ± 2.3 ml/100 g of tissue per min in group IV (4-day survival; 3 of 3, 100%) and 23.5 ± 9.1 ml/100 g of tissue per min in group V (0 of 6, 0%). This was accompanied by graft dehydration during storage and an increased number of erythrocytes in the hepatic sinusoids post-recirculation. We concluded that assessment of liver tissue flow by LDF was very helpful and easy to apply in predicting liver graft failure in the case of preservation with Euro-Collins solution. However, LTBF should be carefully evaluated as a marker of liver graft viability when the liver graft is preserved with mUW.

Key words: Liver transplantation – Liver tissue flow – Laser doppler flowmetry – Euro-Collins solution – Modified University of Wisconsin solution – Liver graft function

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In recent years, there has been growing evidence that the integrity of non-parenchymal cells plays a crucial role in liver graft function after extended cold preservation [1–4]. Cold ischemia damages hapatic sinusoidal endothelium preferentially, which becomes particularly vulnerable when exposed to reflow of the recipient's circulation. Subsequently, the loss of viability of sinusoidal endothelial cells can cause severe microcirculatory disturbances with tissue hypoperfusion, finally compromising parenchymal hepatocyte function, resulting in liver graft failure. In this context, we postulated a correlation between the state of hepatic microcirculation and liver graft viability following hepatic transplantation. Therefore, in this study we investigated whether assessment of hepatic microcirculation is efficacious in predicting early function and survival of liver allografts post-transplantation. To test our hypothesis, we used different periods and solutions for liver preservation. Liver tissue blood flow (LTBF) was assessed by laser doppler flowmetry (LDF).

Materials and methods

Transplantation procedure. We used 23 pairs of female mongrel pigs (18–36 kg) in this study. After intratracheal intubation, liver transplantation was performed under general anesthesia with a mixture of oxygen (2 l/min), nitrous oxide (2 l/min), and fluothane (0.2%). The technique of pig liver allografting has been described in detail elsewhere [5]. Briefly, the donor liver was removed after systemic heparinization (2000 units) by cooling via the portal vein and celiac axis with 1.5 l of preservation solution (4°C). A total of 1.4 l of solution was perfused through the portal vein and 100 ml through the celiac artery. The biliary tree was flushed with 50 ml of solution. The graft was stored in a refrigerator at 4°C. The recipient hepatectomy

Table 1. Outcomes following orthotopic transplantation of liver treated with different solutions and periods of preservation (mean ± SD)

Group	Total ischemia time (h) (n)	Survival (days)	LTBF (ml/100 g tissue per min) (n)	4-day survival (%)
I (EC/4 h)	4.1 ± 0.2 (3)	5, 29, 8	21.2 ± 3.0 (n = 3)	3/3 (100)
II (EC/12 h)	12.6 ± 0.5 (5)	0, 0, 0, 0, 2	10.0 ± 2.8* (n = 4)	0/5** (0)
III (EC-AZA/12 h)	12.6 ± 0.3 (6)	6, 4, 4, 8, 9, 0	19.1 ± 3.4 (n = 6)	5/6 (83)
IV (mUW/4 h)	4.0 ± 0.6 (3)	6, 6, 11	23.0 ± 2.3 (n = 3)	3/3*** (100)
V (mUW/24 h)	24.9 ± 0.6 (6)	0, 0, 0, 0, 0, 0	23.5 ± 9.1 (n = 6)	0/6 (0)

$P < 0.05$, * vs all other groups, ** vs groups I and III and *** vs group V

was carried out with a pump-driven venous bypass connecting the portal and external iliac veins and the external jugular vessel following heparinization (2000 units). The bypass blood flow was maintained above 30 ml/min per kg with a Bio-pump (Bio Medicus, Minn). Arterial and central venous cannulation in the recipient was carried out via a cervial and groin cutdown for monitoring, blood sampling and administration of fluids and medication. Calcium gluconate (850 mg), sodium bicarbonate (40 mEq), and protamine sulfate (10 mg) were administered intravenously (IV) when the blood flow was restored. The donor liver was orthotopically implanted with a suprahepatic vena caval anastomosis (Prolene 4-0), followed by the portal vein anastomosis (cuff technique). Immediately before the completion of the portal vein anastomosis, the graft was flushed via the portal vein with 500 ml of lactated Ringer's solution. Following revascularization, the vena caval anastomosis below the liver (cuff technique) and hepatic artery (Prolene 6-0) was performed. The bile duct was anastomosed by telescoping using a stent. The animals received a constant IV infusion of lactated Ringer's solution (30 ml/kg per h) during surgery and approximately 1000 ml of the same solution postoperatively. They were first fed 24 h after revascularization. One unit of blood was transfused from donor to recipient as the sole coagulation support. Cefamandole (1 g) was given IV daily until day 3.

Experimental protocols. Euro-Collins (EC) and modified University of Wisconsin solutions (mUW) (the additives of original UW solution, adenosine, glutathione, allopurinol, insulin and dexamethasone, and a colloid-5% hydroyethyl starch were omitted) [6] were chosen. The animals were divided into five groups. In group I (EC/4 h, n = 3), the liver graft was stored in EC and implanted 4 h following harvesting. In group II (EC/12 h, n = 5), the liver was stored in EC for 12 h. In group III (EC-AZA/12 h, n = 6), the donor was pretreated with azathioprine (AZA), 1 mg/kg per day, orally (PO) for 3 days, and the graft was preserved as in group II. In group IV (mUW/4 h, n = 3), the liver was preserved in mUW and implanted after harvesting as in group I. In group V (mUW/24 h, n = 6), the liver was transplanted after 24 h of cold storage in mUW.
Parameters and statistics. The 4-day survival rate and liver tissue blood flow (LTBF) were studied as well as graft weight change, histology, coagulation and serum biochemistry. LTBF was measured during donor harvesting and at 1 h after portal venous reflow of the graft with a laser doppler device (model BPM 403A, TSI, St. Paul, Minn.). A representative estimate was the mean of 3 to 6 determinations which were provided by randomly placing the probe in different areas in the same liver. For histology, graft biopsy specimens were stained with hematoxylin and eosin and examined by an independant pathologist (SA). All values were given as mean ± SD. Statistical analysis was made by using the chi-square test or Student's t-test. A P value of less than 0.05 was considered to be statistically significant.

Results

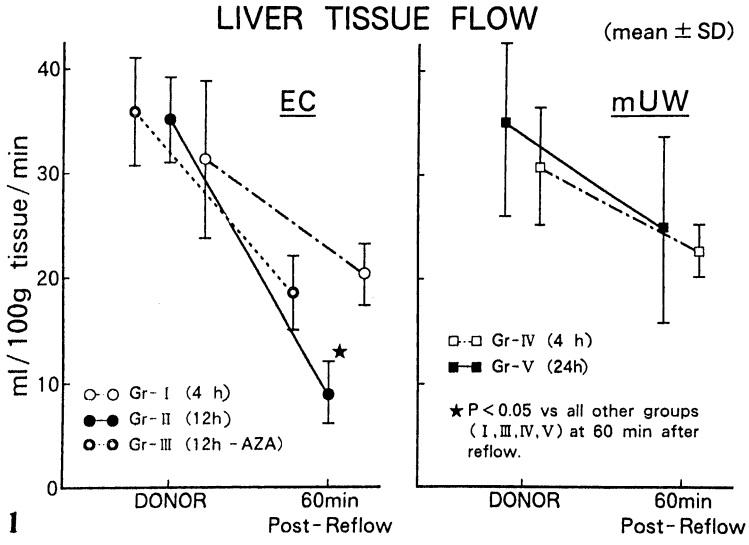
The outcomes of the liver transplants are described in Table 1. All animals receiving transplants approximately 4 h after liver harvesting were alive for more than 5 days (groups I and IV). In contrast, no pig survived beyond 2 days in the EC/12 h (group II) and the mUW/24 h groups (group V). Five of six pigs survived more than 4 days in group III (EC-AZA/12 h) in which the donor was pretreated with AZA. The hepatoprotective effect of AZA pretreatment has been discussed elsewhere [4].

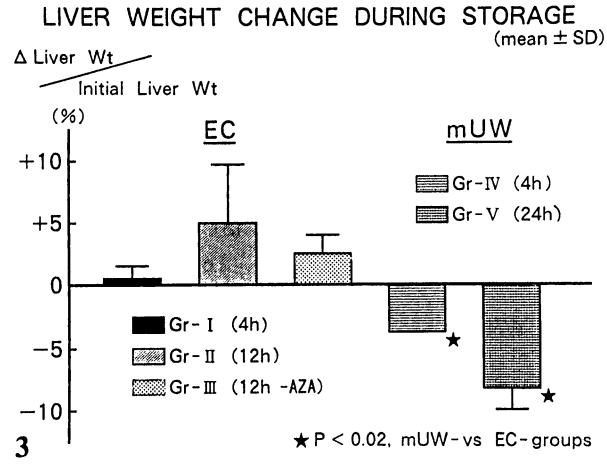
Figure 1 shows the changes in liver tissue flow (LTBF) of the allografts during the transplantation procedure. In the case of storage in EC, the LTBF post-recirculation was significantly higher in the EC-AZA/12 h group (group III), which showed improved animal survival, than that in group II (EC/12 h) which had poor graft survival; all eight grafts (five in group III and all three livers in group I) with LTBF of more than 15 ml/100 g of liver tissue per min functioned well post-implantation. However, all pigs died on the day of surgery in the mUW/24 h group (group V) in spite of a well-maintained flow of the graft tissue.

As shown in Fig. 2, there was no correlation between the transaminase levels and animal survival in the EC groups (groups I, II and III). In the groups treated with mUW, AST was significantly higher in the non-surviving pigs (group V) than that in the pigs with improved survival (group IV). There were no differences in prothrombin values among the groups.

Liver weight changes during cold preservation are shown in Fig. 3. It is of note that the liver grafts in mUW groups (IV and V) lost weight significantly during storage, when compared with the changes in EC groups (I, II and III).

Representative livers at the end of preservation and 1 h after implantation are shown in Fig. 4. As shown in Fig. 4 B and C, EC preservation for 12 h caused substantial swelling of hepatocytes. There was substantial damage to the graft after re-flow of blood for 1 h (eosinophilic and fatty degeneration of hepatocytes, parenchymal haemorrhage, and diffuse neutrophil infiltration in the sinusoids) (Fig. 4 B'). This was significantly ameliorated by AZA





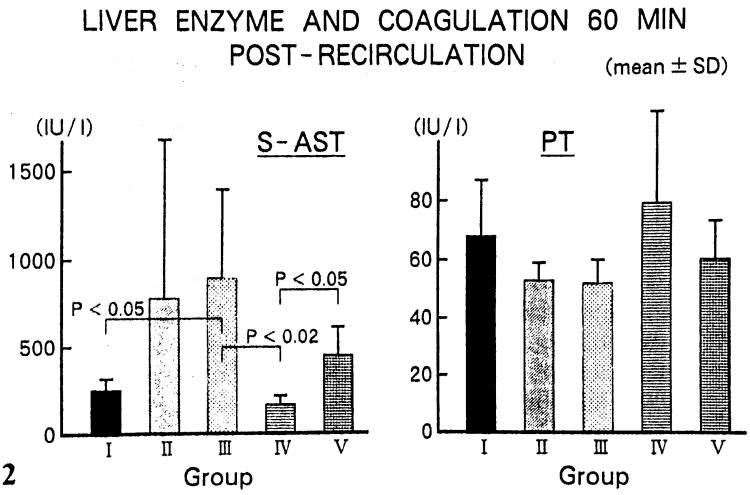


Fig. 1. Tissue flow of the liver graft (LTBF) during harvesting and 60 min after implantation

Fig. 2. Values for serum aspartate aminotransferase (S-AST) and prothrombin time (PT) 60 min after transplantation of the liver graft. The numbers examined in groups I–V were 3, 4, 6, 3, and 6, respectively

Fig. 3. Changes in weight of the liver graft during cold preservation. The numbers examined in groups I–V were 3, 5, 6, 3, and 6, respectively

pretreatment in donors (Fig. 4C'). In comparison with EC-stored liver, mUW storage caused marked shrinkage of parenchymal hepatocytes with subsequent dilatation of sinusoidal spaces (Fig. 4D). An increased number of erythrocytes were found in the sinusoidal lumen at 60 min after transplantation (Fig. 4D').

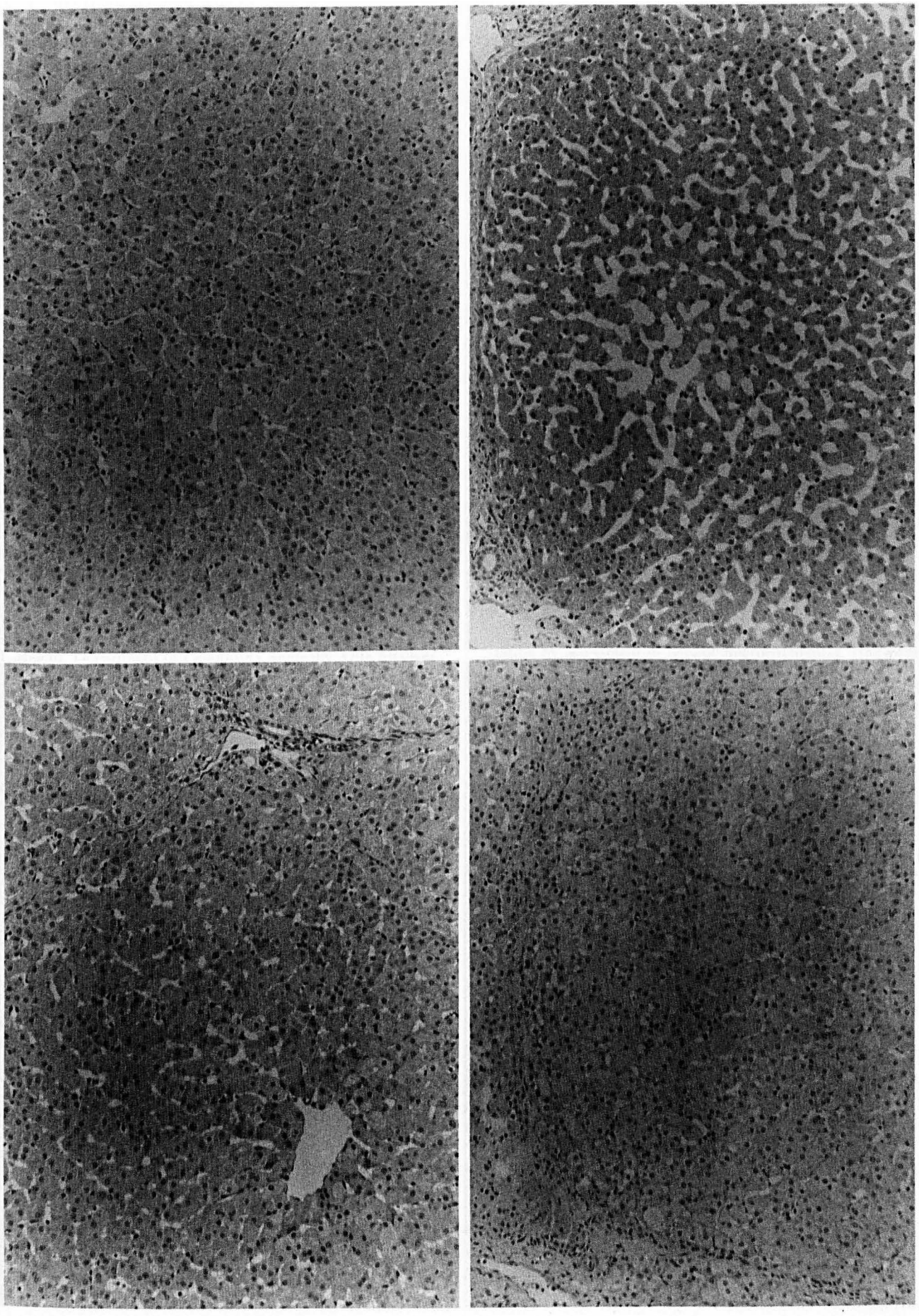
Discussion

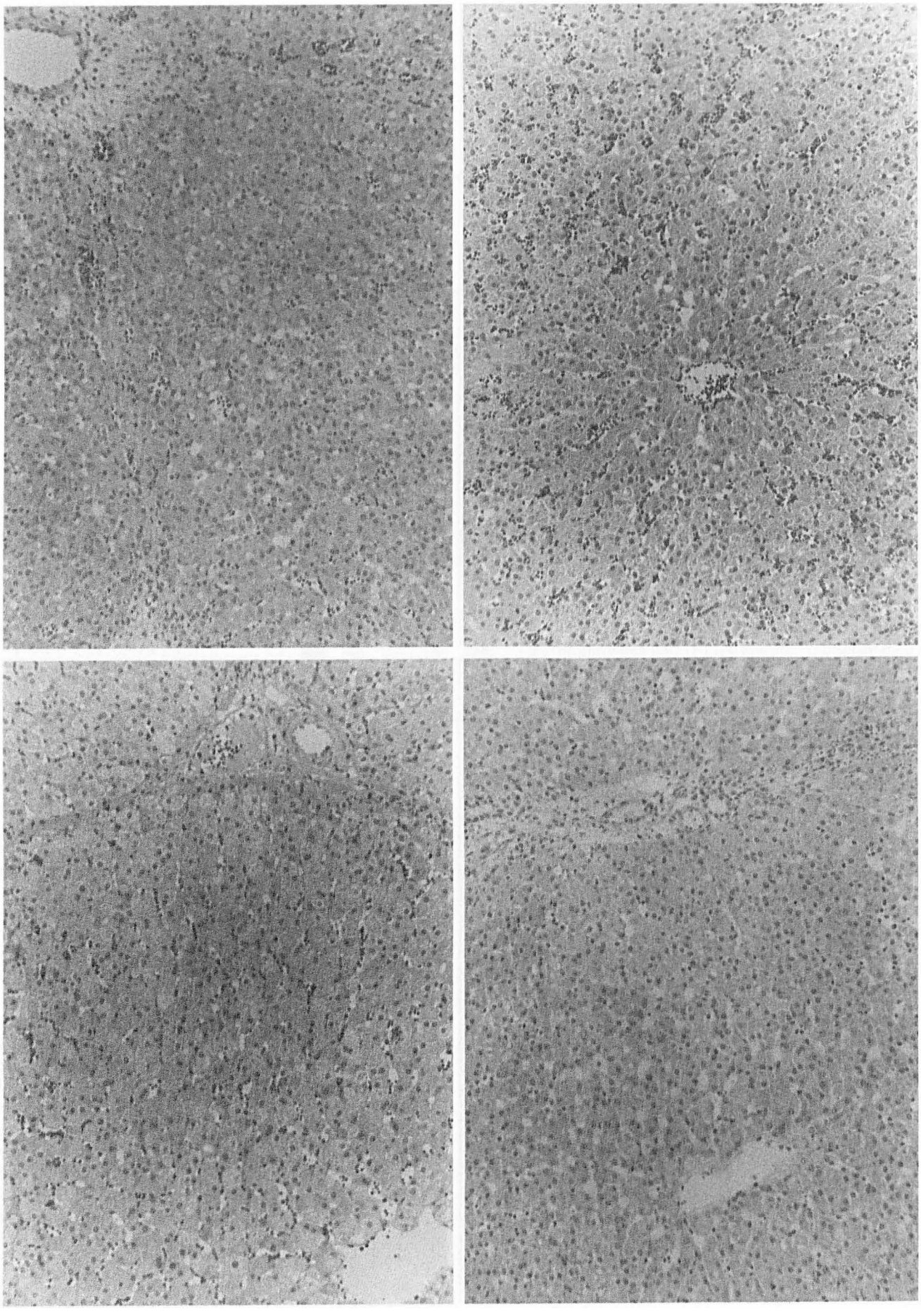
To date, no parameters are available for early, accurate forecasting of liver graft failure; therefore, the patient's condition becomes critical when emergency retransplantation is needed. Biochemical assessment, such as hepatic enzyme release, energy status, and biopsy findings, are unpredictable or time-consuming procedures, and thus not practical on clinical grounds [7–10]. There was no correlation in the present study between animal survival and serum levels of transaminase (AST) when the graft was preserved with Euro-Collins solution.

Hepatic sinusoidal endothelium is the first target tissue facing reflow of the recipient's circulation, therefore, injury to the microvasculature is the primary effect of ischemia/reperfusion insult of the liver graft [1–4]. The parenchymal hepatocytes are subsequently damaged because of disturbances in metabolic exchanges through the sinusoidal endothelium. Indeed, evidence has accumulated that the integrity of the hepatic endothelial cells plays a key role in graft viability, particularly in the case of early ischemia/reperfusion. Therefore, it would be reasonable to speculate that assessment of hepatic microcirculation could be a marker of graft function. In line with this hypothesis, Manner et al. [11] have reported recently that the state of graft tissue perfusion correlates well with preservation damage in pig liver transplantation. They suggest, using H2-clearance technique and HTK solution, that assessment of hepatic microcirculation (30 min post-transplantation) is efficacious in predicting early function and survival of liver grafts.

Our results showed that LTBF was very effective in predicting the viability of liver grafts when preserved with

Fig. 4. Representative livers **A–D** at the end of cold preservation and **A'–D'** 60 min after implantation. **A** and **A'** group I (EC/4 h), **B** and **B'** group II (EC/12 h), **C** and **C'** group III (EC-AZA/12 h), **D** and **D'** group V (mUW/24 h) (Hematoxylin and eosin, x 90)





the Euro-Collins solution; all grafts with LTBF of more than 15 ml/100 g of liver tissue per min functioned well. However, changes in microcirculation of the mUW-stored liver did not correlate with the function of the graft. One explanatation may be the significant graft dehydration induced by mUW. We hypothesize that shrinkage of parenchymal hepatocytes temporarily ameliorated hepatic microcirculation by widening the sinusoidal lumen early during graft recirculation. Another possibility may have to do with our measurement technique (LDF). Since LDF is able to monitor blood flow in a small surface range of liver tissue (1–2 mm^3), it is possible that the small volume of tissue in which LDF measures perfusion is not always representative of total hepatic blood flow [12]. This is of importance in the case of early estimation of the graft recirculation when the state of hepatic microcirculation can be different from site to site or particularly between the deep and surface portion of the allografts. We should be able to estimate more accurately the state of microcirculation if LTBF is evaluated later, in other words, a few hours post-transplantation or at the time of closure of the abdomen. Based on our data, we concluded that assessment of liver tissue flow by LDF is very helpful and easy to apply for predicting liver graft failure in the case of Euro-Collins preservation. However, LTBF should be carefully evaluated as a marker of liver graft viability when the liver graft is preserved in mUW.

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